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Dietz, MW; Piersma, T; Dekinga, A

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BODY-BUILDING WITHOUT POWER TRAINING: ENDOGENOUSLY REGULATED PECTORAL MUSCLE HYPERTROPHY IN CONFINED SHOREBIRDS

MAURINE W. DIETZ[1,*], THEUNIS PIERSMA[1,2] AND ANNE DEKINGA[2]

[1]*Centre for Ecological and Evolutionary Studies, Zoological Laboratory, University of Groningen, PO Box 14, 9750 AA Haren, The Netherlands* and [2]*Netherlands Institute for Sea Research (NIOZ), PO Box 59, 1790 AB Den Burg, Texel, The Netherlands*

*Author for correspondence (e-mail: m.w.dietz@biol.rug.nl)

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Summary

Shorebirds such as red knots *Calidris canutus* routinely make migratory flights of 3000 km or more. Previous studies on this species, based on compositional analyses, suggest extensive pectoral muscle hypertrophy in addition to fat storage before take-off. Such hypertrophy could be due to power training and/or be effected by an endogenous circannual rhythm. Red knots of two subspecies with contrasting migration patterns were placed in a climate-controlled aviary (12 h:12 h L:D photoperiod) where exercise was limited. Using ultrasonography, we measured pectoral muscle size as the birds stored fat in preparation for migration. At capture, there were no differences in body mass and pectoral muscle mass between the two subspecies. As they prepared for southward and northward migration, respectively, the tropically wintering subspecies (*C. c. canutus*) gained 31 g and the temperate wintering subspecies (*C. c. islandica*) gained 41 g. During this time, pectoral mass increased by 43–44 % of initial mass, representing 39 % (*C. c. canutus*) and 29 % (*C. c. islandica*) of the increase in body mass. The gizzard showed atrophy in conjunction with a diet change from molluscs to food pellets. Although we cannot exclude the possibility that the birds' limited movement may still be a prerequisite for pectoral muscle hypertrophy, extensive power training is certainly not a requirement. Muscle hypertrophy in the absence of photoperiod cues suggests the involvement of an endogenous circannual process.

Key words: pectoral muscle, stomach, migration, intra-individual variation, use–disuse mechanism, endogenous annual rhythm, red knot, *Calidris canutus*.

Introduction

Widely accepted mechanisms enabling adaptive changes in muscle strength and size with changing work loads are power and endurance training, although this is mainly based on mammalian studies (e.g. Goldberg et al., 1975; Goldspink, 1991; Kannus et al., 1992; Antonio and Gonyea, 1993; Kelley, 1996). Power training of pectoral muscles was invoked to explain the inter-individual allometry of pectoral muscle mass on body mass in Cooper's hawks *Accipiter cooperii* (Marsh and Storer, 1981): an increased body mass would lead to an increased work load since wing area remains constant. Similar arguments were invoked in discussions of changes in pectoral mass in long-distance migrating birds such as red knots *Calidris canutus* (Davidson and Evans, 1988). Although power training might be responsible for some of the changes observed in avian migrants (Marsh, 1984; Driedzic et al., 1993), there are field examples of muscle hypertrophy in which the behaviour patterns thought to be necessary for extensive power training are hard to identify. A case in point is birds that moult all their flight feathers at once. Despite their flightlessness, the pectoral muscle mass of such waterfowl increases at the end of the moulting period (e.g. Ankney, 1984; Austin and Fredrickson, 1987; Piersma, 1988; Jehl, 1988; Thompson and Drobney, 1996; Brown and Saunders, 1998). Pectoral muscle growth may even coincide with a loss of fat stores and therefore a decrease in body mass (Jehl, 1997; Brown and Saunders, 1998).

Endogenous circannual processes play an essential role in the control of seasonal migrations (e.g. Berthold, 1990; Gwinner, 1990, 1996). Circannual rhythms have been shown to underlie the timing of migration, preparatory fat deposition and moulting, migratory restlessness, orientation direction, food preference and the morphology of the alimentary tract (e.g. Berthold, 1974; Gwinner and Wiltschko, 1980; Gwinner, 1987, 1990, 1996; Bairlein, 1990). Such circannual rhythmic processes may also play a role in the changes in pectoral muscle size during migration, whether or not complemented by 'instantaneous' power training mechanisms.

A long-standing problem in studies of adaptive changes in organ size is the fact that individuals cannot be sampled more than once (Lindström and Piersma, 1993; Van der Meer and Piersma, 1994; Piersma and Lindström, 1997). Using modern visualisation techniques such as nuclear magnetic resonance

imaging and ultrasound scanning (Fuller et al., 1994; Haefner et al., 1996; Dietz et al., 1999), it is now possible to document changes in organ size in living animals. A calibration study on red knots using ultrasonography enabled us to measure pectoral muscle size with a point-accuracy of approximately 25 % and stomach size with an accuracy of 35 % (Dietz et al., 1999). Sequential measurements of individuals by a single observer would further reduce this variation.

The present study capitalises on this technical development and the availability of two contrasting populations of a long-distance migrating shorebird, the red knot. In the Dutch Wadden Sea in August, two subspecies of red knots occur (Piersma et al., 1993a): (1) *Calidris c. islandica*, which breeds in high arctic Greenland and north-east Canada and winters in the Wadden Sea and other intertidal areas of western Europe (Davidson and Wilson, 1992), and (2) *C. c. canutus*, which breeds in high arctic parts of western Siberia and winters in tropical West Africa (Piersma et al., 1992). Thus, at this place and time we find together birds that prepare (by going into wing moult) to winter in a temperate climate (*C. c. islandica*) and birds that prepare (by rapidly storing fuel) to take off for a 4500 km flight to West Africa (*C. c. canutus*). Individuals of both groups were captured, placed in a climate-controlled aviary and kept under a constant light:dark photoperiod of 12 h:12 h, where they continued to moult and to store fuel (see, for example, Piersma et al., 1995). We measured body mass and moult over time until April of the following year when, in the field, both subspecies prepare for spring migration towards the breeding grounds and moult into their summer plumage. We determined the size of the pectoral muscles and gizzard using ultrasound immediately after capture and at three times during captivity in both groups.

Materials and methods

Experimental arrangement

Eight wild red knots *Calidris canutus* were captured with mist-nets on 5 August 1997 in the Dutch Wadden Sea. The birds were weighed, and the moults of the body feathers and primaries, as well as the extent of breeding plumage, were scored (Piersma and Jukema, 1993). One observer (A.D.) measured left pectoral muscle thickness (±0.1 mm) and gizzard diameter (±0.1 mm) using ultrasonography (Pie 200, 7.5 MHz linear probe; Pie Medical Benelux BV, Maastricht, The Netherlands). The birds were taken to a climate-controlled aviary (4 m×2.5 m×2.5 m) at NIOZ, Texel, The Netherlands. Ambient temperatures ranged from 16 to 19 °C, and the birds were subjected to a constant light:dark photoperiod of 12 h:12 h. The red knots had access to a small barren artificial mudflat in which they often practiced probing. Trout pellets (Trouvit, Produits Trouw, Vervins, France) and fresh water were available *ad libitum*. Plumage, primary moult and body mass were determined weekly. Birds were assigned to subspecies on the basis of the onset of primary moult (Fig. 1E,F); in *C. c. islandica*, the moult started in August immediately after capture (four of eight birds). As is typical for *C. c. canutus* (Piersma et al., 1996), the other four birds postponed wing and body moult for half a year and in the meantime showed considerable fuel storage for southward migration, a flight that was eventually not performed. Pectoral muscle thickness and stomach diameter of the red knots were determined after 86 (late October), 206 (early March) and 261 days (late April) in captivity. This period was too short to include the fuel storage episode for northward flight in *C. c. canutus*.

Ultrasound measurements

Pectoral muscle thickness (±0.1 mm) was measured by placing the ultrasound probe transversely on the left pectoral muscle at an angle of approximately 90 ° from the rostral top of the sternum to the shoulder, i.e. the joint of the coracoid with the clavicula (see Fig. 1 in Dietz et al., 1999). This resulted in white 'V'-shaped images of the keel of the sternum and the coracoid in which the breast muscle is located. The thickness was measured from the bottom of the 'V' to the top of the muscle (see Fig. 2 in Dietz et al., 1999). In general, very little or no subcutaneous fat was present at the site where the pectoral muscle was measured. Noting that, in mollusc-eating sandpipers the proventriculus is very small and the stomach consists almost entirely of the muscular part (gizzard; Piersma et al., 1993b), the diameter of the gizzard (±0.1 mm) was measured by placing the probe transversely on the belly of the bird at an angle of approximately 45 ° just below the sternum (see Fig. 2 in Dietz et al., 1999). The gizzard was visible as a roundish, slightly elliptical, image. Both the horizontal (stomach width) and vertical (stomach height) diameters were measured (see Fig. 2 in Dietz et al., 1999). The entire ultrasonographic examination took in total approximately 10 min per bird. The scanning gel was easily and completely removed from the feathers using lukewarm water, and the birds remained in perfect condition after the procedure.

Observer-specific (A.D.) calibration curves were made to determine the relationship between the appropriate ultrasound measurement and total pectoral muscle mass (left and right side) or stomach mass, following the method described by Dietz et al. (1999). For this, a group of 22 dead red knots (composition similar to that in Dietz et al., 1999) was used in which large variations in pectoral and stomach mass were present. The following predictive equations were obtained (see Fig. 2):

$$M_p = -10.93+31.73W_p \ (r^2=0.797,\ P<0.001), \quad (1)$$

and

$$M_s = -5.35+7.88W_s \ (r^2=0.700,\ P<0.001), \quad (2)$$

where M_p is total pectoral muscle mass (g), W_p is pectoral muscle thickness (cm), M_s is stomach mass (g) and W_s is stomach width (cm). Stomach width was used to estimate stomach mass, because in live red knots this appeared to be the most reliable measurement of the stomach diameter. Using these equations, total pectoral muscle mass and stomach mass of the live knots were estimated.

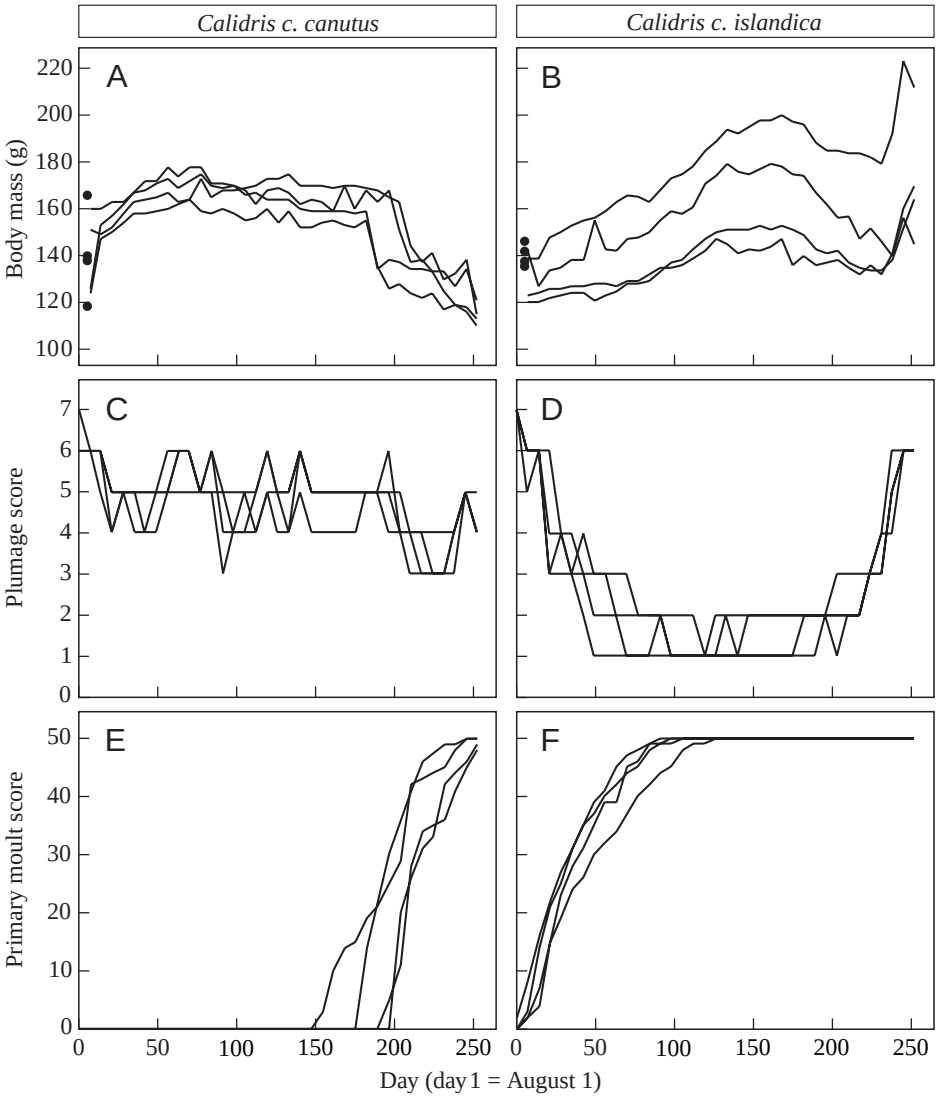

Fig. 1. Change in body mass (A,B), plumage score (C,D) and flight feather (primary) moult score (E,F) in eight red knots of two subspecies kept under constant conditions in a single aviary (L:D 12 h:12 h, ambient temperature 16–19 °C). Each line represents an individual bird. Plumage scores vary between 1 (full winter plumage) and 7 (full summer plumage). Primary moult scores vary between 0 (all old feathers) and 50 (all new feathers).

Statistics

Mean values of two groups were compared using a Student's *t*-test or a paired Student's *t*-test when appropriate. Calibration equations and the relationship between pectoral muscle mass and body mass were calculated using least-squared linear regression. Differences between linear regression lines were investigated using multivariate analysis of variance (MANOVA). Repeated-measures analysis was used to test whether body mass or organ mass varied with time within individuals, and to test whether the variation over time differed between subspecies. Repeated-measures analysis was also used to test within a subspecies whether the variation over time in pectoral or stomach mass differed from that in body mass. For this, the data were normalised per individual by dividing the measurements by the value of the initial measurement at capture (i.e. value at day i/value at day 0). All statistical analyses were performed using SPSS 8.0 for Windows.

Results

The body masses of the four *C. c. islandica* showed a pattern with two peaks, a minor peak in midwinter and a major peak in April in preparation for spring migration (Fig. 1B). During this latter peak, they moulted their body feathers and went into a breeding plumage. The primaries were moulted immediately after capture (Fig. 1D,F). The body masses of the four *C. c. canutus* showed a peak only in autumn (Fig. 1A). The mass peak for northward migration (Piersma et al., 1996) did not occur in the time period investigated. *C. c. canutus* did not complete the body moult. The primaries were moulted in winter, after the autumn peak in body mass (cf. Piersma et al., 1996; Fig. 1C,E).

Ultrasound measurements were taken at four time points, and in the analysis below only data obtained at those points are taken into account. Calibration curves are shown in Fig. 2. At capture, body mass and estimated pectoral mass did not differ between the subspecies (Fig. 3A,B, Student's *t*-test, both $P>0.05$). During fat storage, substantial increases in body mass and pectoral mass occurred. Within individuals, the variation over time in both body mass and pectoral mass was significant. This variation differed significantly between subspecies (Table 1) because of the differences in timing. In preparation for their

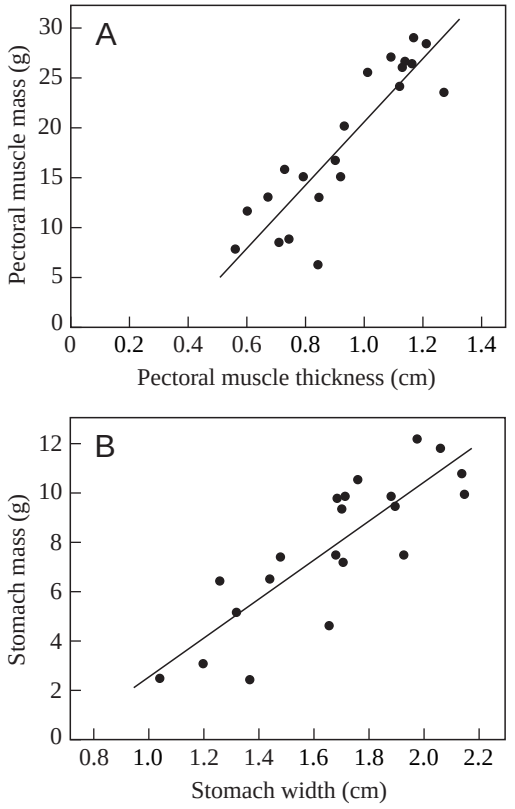

Fig. 2. Calibration curves for ultrasonographic measurement of left pectoral muscle thickness and true overall pectoral muscle mass (left and right side) (A), and for ultrasonographic measurement of stomach width and true stomach mass (B). For equations, see text.

Table 1. *Repeated-measures analysis of variance results for changes in body mass, estimated pectoral muscle and stomach mass over time in relation to subspecies*

	N	Source of variation	*F*	*P*
Body mass	4, 4	Subspecies	26.865	<0.0001
		Time	3.772	0.029
		Subspecies × time	3.105	0.129
Pectoral mass	4, 4	Subspecies	9.970	<0.0001
		Time	9.861	<0.0001
		Subspecies × time	0.707	0.433
Stomach mass	3, 3	Subspecies	2.437	0.115
		Time	55.803	<0.0001
		Subspecies × time	1.658	0.267

N, number of birds for each subspecies: *Calidris c. canutus, C. c. islandica*.

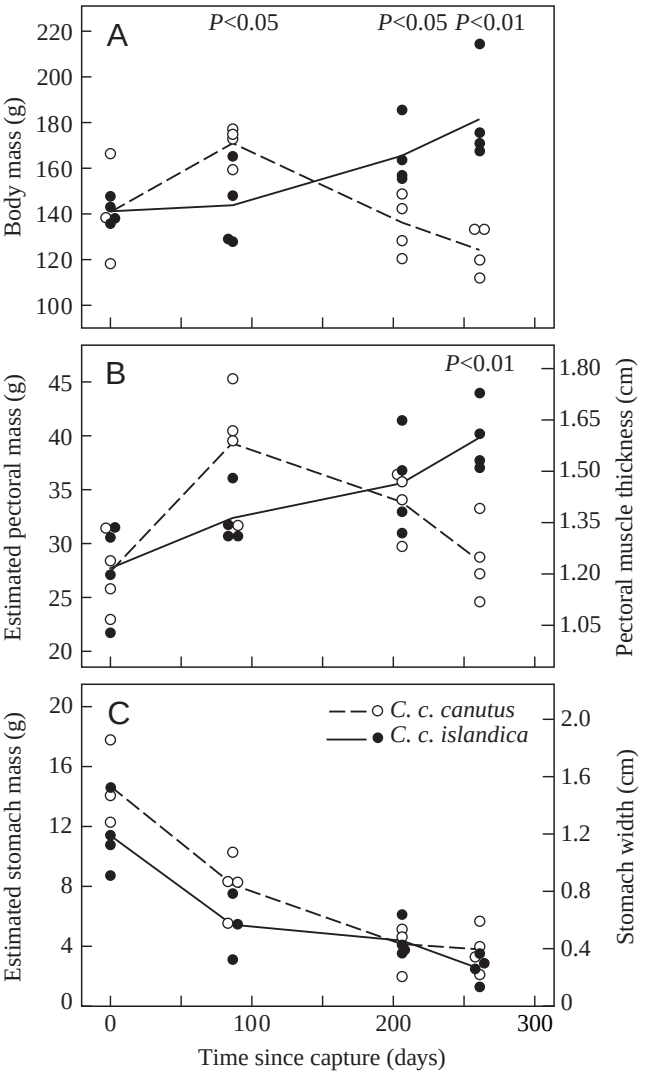

Fig. 3. Change in body mass (A), estimated pectoral muscle mass and measured pectoral muscle thickness (B) and estimated stomach mass and measured stomach width (C) in eight red knots of two subspecies kept under constant conditions in a single aviary (L:D 12 h:12 h, ambient temperature 16–19 °C). Significant differences between the subspecies for a specific day are indicated by the *P*-values at the top of the panels. Open symbols, *Calidris canutus canutus*; filled symbols, *C. c. islandica*.

southward migration, *C. c. canutus* had gained 31 g in body mass by day 86 (22 % of initial mass). This increase in body mass included a 12 g increase in pectoral mass (44 % of initial pectoral mass). In contrast, *C. c. islandica* gained little mass (1.5 g) over this period and showed no changes in pectoral mass (paired Student's *t*-test, both $P>0.05$). The difference in pectoral masses between the subspecies was marginally significant at day 86 ($t_6=2.25$, $P=0.066$). After the autumn migration peak, body mass and pectoral mass of *C. c. canutus* decreased towards initial levels, while *C. c. islandica* started to gain body mass to prepare for northward migration. At day 261, the body mass of *C. c. islandica* had increased by 41 g compared with the initial mass (29 %). During the same period, the pectoral muscles had gained 12 g (43 % of initial mass) and were significantly larger than those of *C. c. canutus* ($t_6=-4.70$, $P<0.01$). The increases in body mass and pectoral mass in *C. c. islandica* in preparation for northward migration did not differ from those of *C. c. canutus* in preparation for southward migration (Student's *t*-test, both $P>0.05$).

The increase in pectoral mass contributed a considerable part of the overall increase in body mass (39 % for *C. c. canutus*

Table 2. *Repeated-measures analysis of variance results testing to whether the variation in estimated pectoral muscle and stomach mass over time differed from that in body mass for each subspecies*

	N	Source of variation	*F*	*P*
Calidris c. canutus	4, 4	Component	2.206	0.123
Body mass *versus*		Time	16.331	<0.0001
pectoral mass		Component × time	3.007	0.134
Calidris c. canutus	4, 3	Component	34.886	<0.0001
Body mass *versus*		Time	49.110	<0.0001
stomach mass		Component × time	57.170	0.001
Calidris c. islandica	4, 4	Component	1.060	0.391
Body mass *versus*		Time	16.410	<0.0001
pectoral mass		Component × time	1.289	0.300
Calidris c. islandica	4, 3	Component	49.450	<0.0001
Body mass *versus*		Time	14.161	<0.0001
stomach mass		Component × time	82.235	<0.0001

N, number of birds for each component: body mass, organ mass.

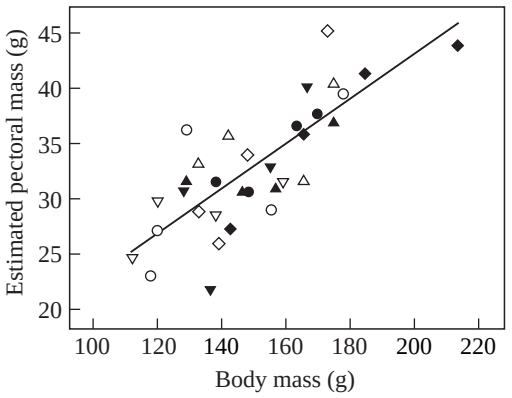

Fig. 4. The relationship between estimated pectoral muscle mass and body mass in eight red knots of two subspecies. Each symbol represents an individual bird; open symbols, *Calidris canutus canutus*; filled symbols, *C. c. islandica*. The solid line represents the linear regression equation through the data: y=2.36+0.20x, N=32, r^2=0.63 g, P=0.

and 29 % for *C. c. islandica*). This is reflected by the close relationship between body mass and pectoral muscle mass. Within a subspecies, the variation in pectoral mass over time did not differ from the variation in body mass (Table 2). This is even more obvious when pectoral mass is plotted against body mass (Fig. 4). The rate of increase in estimated pectoral mass with body mass (0.20±0.03g g^{-1}, mean ± S.E.M., N=32) did not differ from that between pectoral mass and body mass in a large sample of dissected red knots from the Wadden Sea (0.21±0.01g g^{-1}, mean ± S.E.M., N=151; T. Piersma, unpublished data; MANOVA, $F_{1,179}$=0.003, P>0.05).

As for body mass and estimated pectoral mass, estimated stomach mass did not differ between the subspecies at capture (Fig. 3C, t_5=0.85, P>0.05). Stomach mass varied significantly over time within individuals, but this variation did not differ between subspecies (Table 1). In all birds, stomach mass decreased rapidly over time until it reached a level of only approximately 25 % of the mass at capture. Therefore, the pattern of variation in stomach mass over time differed strongly from that in body mass (Table 2).

Discussion

Methodological caveats

Because red knots can store large amounts of abdominal and subcutaneous fat (Piersma et al., 1999), the deposition of subcutaneous fat on the pectoral muscles could, in principle, have interfered with the measurements of pectoral muscle thickness: it could have yield erroneously high measurements of breast muscle thickness. However, subcutaneous fat is not equally distributed over the pectoral muscles. At the measuring site, subcutaneous fat shows up only during the last part of the preparation for migration and generally only as a very thin layer (M. W. Dietz and A. Dekinga, unpublished observations). Therefore, it is unlikely that subcutaneous fat storage has resulted in an overestimate of pectoral muscle thickness. Another error might result from the storage of fat inside the pectoral muscles. In a sample of red knots (*C. c. islandica*) preparing for a northward flight in Iceland, the fat content of the pectoral muscle showed a marginal increase from 5.61±1.86 % to 6.44±1.52 % (means ± S.D., N=15 and N=12, respectively; see Piersma et al., 1999). If our birds had shown similar increases in pectoral muscle mass due to internal fat storage, overall pectoral mass would have increased by approximately 2.06 g. This is only 7.5 % of the initial pectoral mass at day zero of approximately 27.5 g, which is much lower than the increase actually measured (on average 43 %).

Adaptive stomach atrophy

In wild red knots, stomach mass decreases prior to take-off on long-distance flights (Piersma et al., 1993b; Piersma and Lindström, 1997; Battley and Piersma, 1997) and increases again after arrival at the stopover areas or wintering grounds (Piersma et al., 1999). In all captive red knots, the stomach showed a severe atrophy; in total, a size reduction of approximately 75 %. Stomach hypertrophy did not occur. The atrophy was probably attributable to the change in diet associated with the switch from the wild, where the birds foraged mainly on small bivalves which they ingested whole and cracked in their stomach, to captivity, where they ate soft food pellets (Piersma et al., 1993b). This diet switch reduces the work load on the muscular stomach and this will induce stomach atrophy, just as inactivity induces atrophy in skeletal muscles (e.g. Goldspink, 1991; Kannus et al., 1992).

Such an effect of changes in diet on stomach size has been demonstrated previously in mallards (*Anas platyrhynchos*; Kehoe et al., 1988) and Japanese quail (*Coturnix coturnix japonica*; Starck, 1999). In both species, a change between diets with high or low fibre content resulted in reversible changes in stomach mass. In both mallard and quail, stomach size increased or decreased, depending on the direction of the switch, by approximately 50 % within 2 weeks after the diet

switch (Kehoe et al., 1988; Starck, 1999). The frequency of our measurements was too low to determine when most of the stomach atrophy occurred. However, the mallard and quail studies suggest that the major stomach size changes would have occurred shortly after capture.

Thus, variation in stomach mass in red knots may not be regulated by endogenous circannual processes, but according to use–disuse (hypertrophy–atrophy) mechanisms (Piersma et al., 1993b). Nevertheless, it is possible that, in the wild, stomach mass is indirectly regulated by a circannual rhythm through a shift in food preference. In a number of migrant bird species, especially passerines but also some shorebirds and geese, striking seasonal diet selection shifts have been observed (Berthold and Berthold, 1973; Berthold, 1974; Bairlein, 1990). Bairlein (1990) suggests that these seasonal diet shifts are associated with an endogenous circannual change in food preference.

Muscle hypertrophy without power training?

Severe skeletal muscle atrophy is induced by inactivity and immobilisation (e.g. Goldspink, 1991; Kannus et al., 1992). Even though the captive conditions allowed rather limited flight activity (a maximum of a few minutes of hovering per day) compared with the minimum of 2 h of flight in free-living red knots during the non-breeding season (Piersma et al., 1993a), some pectoral muscle atrophy might have been expected. Yet, during the 8 month period in captivity, the observed pectoral muscle mass never decreased to levels below the initial mass (Fig. 3B). Moreover, during preparation for migration, pectoral mass increased on average by 43 %, which is unlikely to have been induced by an increase in work load or power training. Under the constant conditions in captivity, the red knots showed rather normal annual variation in body mass, and the variation in pectoral muscle mass resembled the normal pattern of pectoral muscle mass variation in free-living red knots. Extensive power training is not a requirement for pectoral muscle hypertrophy to occur.

The increase in pectoral muscle mass of the captive red knots during pre-migratory preparation does not imply that muscle size adjustment is the only requirement for 4500 km flights. In blue-winged teal (*Anas discors*), the increase in pectoral muscle mass in the last part of wing moult is accompanied by a rapid increase in citrate synthase activity (Saunders and Klemm, 1994). This increase in aerobic capacity occurred without aerobic conditioning because the birds were behaviourally flightless, which suggests that the process was endogenously controlled. Red knots that had been in captivity for more than half a year were able to fly for 10 h in a wind tunnel at speeds of 50 km h^{-1} after only a few training sessions to familiarise the birds with the highly artificial circumstances (Å Lindström, A. Kvist, T. Piersma, A. Dekinga and M. W. Dietz, in preparation), indicating that, even in a flight-limiting situation such as captivity, red knots are able to maintain an appropriate level of power output of their pectoral muscles. This suggests endogenous regulation.

An endogenous circannual timer?

The patterns of variation in body mass, plumage and primary moult in our captive red knots resembled those found in the wild (Piersma et al., 1996, 1999) and can be interpreted accordingly. The birds were kept under a constant photoperiod, and the variation found must therefore have been regulated by an endogenous circannual rhythm (Gwinner, 1990, 1996; Cadée et al., 1996). Although the variation in pectoral muscle mass resembled the pattern found in the wild (Battley and Piersma, 1997; Piersma et al., 1999), the variation in stomach mass differed considerably from that in free-living red knots (Piersma et al., 1993b). This suggests that size variation in the two muscular organs occurs by two different mechanisms: endogenous processes and power training (a use–disuse mechanism), respectively.

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